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PATHOTYPING OF SELECTED HYBRID RICE VARIETIESBY USING TWO SETS OF DIFFERENTIALS

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ABSTRACT

Hybrid rice lines are highly susceptible to many diseases and insect-pests (Reddy et al., 1996). Among these biotic stresses, bacterial blight is one of the most devastating diseases in the rice growing areas. To know the virulence analysis of pathogen, 30 hybrid rice varieties having their CMS, restorer, maintainer lines and inbred cultivars disease leaf samples were collected, isolated and pathotyped by using both the national cultivar differentials and near isogenic lines (NILs) for the present study. Pathotyping data obtained from NIL and cultivar differentials revealed the possibility of deploying them for enhancing the resistance against bacterial blight disease of the rice hybrids.

KEYWORDS: Hybrid Rice-Bacterial Blight-Pathotyping-Cultivar Differentials-Near Isogenic Lines.

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Original Article

INTRODUCTION

Hybrid rice lines are highly susceptible to many diseases and insect-pests (Reddy et al., 1996) and bacterial blight is one of the devastating constraints in neutralizing their high yield potential. In an effort to understand whether the bacterial blight pathogen isolates infecting inbred cultivars are different from those affecting hybrid lines, their CMS, restorer, maintainer lines, set of 30 isolates (Sirisha. CH. & Srinivas Naik. K. 2021) of X anthomonas oryzae pv. oryzae was obtained from these lines grown in the experimental fields of NRRI during dry season. Incorporating resistance to bacterial blight in hybrid varieties of rice is a prioritized area for present study (Chen et al., 2000). Pathotyping data is helpful to the plant breeders to deploy resistance genes in the respective local varieties.

REVIEW OF LITERATURE

Since, so long the progression of infestation of bacterial blight disease caused by Xanthomonas oryzae pv. oryzae is not clearly known to the pathologists in the world. On the contrary, the virulence capacity of pathogen within the region and across the countries is able to study by them. A new virulent strain was observed and suspected to be cause of evolution during the year 1957, in Japan (Kuhara et al., 1965). Xa21 has been one of the most preferred genes for improving resistance in rice against bacterial blight. Xa21 has been introgressed either singly or in combination with other genes. However, ineffectiveness of IRBB 21 carrying Xa21 to resist bacterial blight in a few locations in India (DRR 2002, Lavanya et al., 1998, Goel et al., 1998) and in Indonesia (Bustamam et al., 1996) has been reported (C. Sirisha et al., 2004).

Incorporating resistance to bacterial blight in hybrid lines is a priority area (Chen et al., 2000). He successfully deployed resistance gene Xa-21 into a most widely used restorer line Minghui 63 in China with a

view to improve its resistance as well as to generate a resistant hybrid line.

MATERIALS AND METHODS

Plant Material Collected for this Study

Near isogenic lines (NILs) seed material having of single and multiple gene combinations were used for this study. 30 varieties of hybrid rice varieties of CR 1014, CR 749-20-2 (2), CR 679-2, CR 839, CR 2007, IET 16645-Inbred culture; IR 62829A (2), CRMS 31A, CRMS 32A-CMS line; IR 62829B-Maintainer line; IR 53258, IR 42266-Restorer lin; RH 10-Pusa hybrid; PA 6201-Proagro hybrid; HKRH 1008-Indo-American hybrid; MPH 525-Mahyco hybrid; PAC 89001-ITC hybrid; PHB 71 (2)-PAU hybrid; CRHR 1, CRHR 4 (3), CRHR 5-CRRI hybrid; DRRH 1-NRRI hybrid; MTURH 2020-Maruteru hybrid andNSD-2 (2)-NDUAT were collected from NRRI research plots.

Seeds of near isogenic lines carrying bacterial blight resistance genes singly and in different combinations IRBB 3 (Xa-3), IRBB 4 (Xa-4), IRBB 5 (xa-5), IRBB 7 (Xa-7), IRBB 10 (Xa-10), IRBB 13 (xa-13), IRBB 21 (Xa-21) (Ogawa, 1993; 1996), were obtained from the International Rice Research Institute, Philippines. 6 National cultivars, Cempo Selak (Xa-3), IR 20 (Xa-4), DV 85 (xa-5, Xa-7), IR 8 (Xa-11), Java 14 (Xa-1, Xa-3, Xa-12 and Xa-hg), and BJ 1 (xa-13) (Horino et al., 1981; Busto, 1991) seeds were collected from the National Rice Research Institute, Hyderabad, India.

Modified Wakimoto's Semi-Synthetic Medium (wf-p) Composition

0.5 gm of Calcium nitrate [$Ca(NO_3)2$], 1.82 gm of disodium hydrogen phosphate [Na_2HPO_4], 20.0 gm of sucrose [($C_{12}H_{22}O_{11}$)], 5.0 gm of peptone(bacteriological), 0.05 gm ferrous sulphate [$FeSO_4$], 1.0 lit of distilled water (dH_2o) and the medium was adjusted to 6.8-7.2 pH before sterilization.

Composition of Skimmed Milk Medium

100.0 gm; skimmed milk powder, 5.0 gm; mono-sodium glutamate, 1.0 lit; distilled water and the medium was adjusted to 6.5 pH before sterilization. Isolation and maintenance of bacterial leaf blight pathogen is done by using semi synthetic modified media of Wakimoto' (WF-P) (Karaganilla et al., 1973) and for long term storage, the bacterial culture of 48 hrs. is stored in skimmed milk medium in refrigerator at 4 ^{0}C for further use (Nelson et al., 1994).

Preparation of Inoculum

To the Petri-plate, both 48-72 hr. old X. oryzae pv. Oryzae culture and sterile distilled water was added. The bacterial colonies were scraped and suspended in sterile distilled water. The cell suspension is mixed well and the O.D. is maintained to 1.0 (10^8 CFU/ml).

Method of Inoculation

To test the rate of resistance and susceptibility reaction in the rice varieties, clip-inoculation method is used. Seedlings of 21-day-old hybrid and their CMS, restorer and maintainer rice varieties were taken and clip-inoculated with 48-hr- X. oryzae pv. oryzae (1.0 O.D) cell suspension culture. A pair of clippers dipped in the bacterial cell suspension culture was used to cut the First and second leaves from the top of the rice plant (Kauffman et al., 1973). To know the scale of action in strain-cultivar, three to four leaves of each plant from four plants were inoculated. Each test was repeated thrice. The plants were maintained in a galvanized iron wire net house under natural photoperiodic conditions. Infected leaves were collected after 7-10 days.

Observations on Disease Development

With the help of ruler, diseased lesion length was measured from the point of inoculation to bottom of the leaf. Data was recorded by taking the grand mean from the triplets. Each mean having three replicas. If the diseased lesion length was measured from 0 to 5cm in the scale, it was noted as a resistant reaction. Otherwise more than 5cm diseased lesion length was considered it as susceptible reaction.

RESULTS

The Pathogen Virulence Analysis

To analyze the virulence, Pathotyping is done for X. oryzae pv. oryzae isolates with the help of two sets of differentials. The virulence patterns of 30 hybrid rice varieties are studied by using both the national cultivar differential set as well as the NIL differentials set. These isolates were grouped into a total of four pathotypes, xa-1, xa-3, xa-5 and xa-9 (Figure 1) when pathotyped using the national cultivar differentials (Table 1). Pathotype xa-1 consisted of a maximum of 27 isolates. However, when all the 30 isolates were pathotyped using the NIL differentials, seven pathotypes, XA-1, XA-2, XA-5, XA-6, XA-8, XA-21, and XH-1 were detected (Figure 2) (Table 2). The pathotype XA-6 consisted of a maximum of 12 isolates.

Similar to the diversity showed by DNA strains when the differential set consisting of near-isogenic lines carrying single resistance genes was used. Virulence data obtained with NIL differentials revealed that all the isolates were compatible with the resistance genes Xa-3 and Xa-4.

Clear cut differences between the pathogen isolates originating from inbred rice varieties/cultures and from the hybrid rice varieties and their CMS, restorer and maintainer lines could not be observed with the national cultivar set of differentials. However, the pathotypes were incompatible with the genes, xa-5, Xa-10, xa-13 and Xa-21suggesting the possibility of deploying them for enhancing the resistance of the rice hybrids tested.

The fingerprints of Xanthomonas oryzae pv. oryzae isolates numbering 30 obtained from hybrid lines, their CMS, restorer and maintainer lines, revealed a greater amount of genetic diversity. Fifteen lineages were detected at a similarity level of 60%. These isolates were grouped into a total of four pathotypes, xa-1, xa-3, xa-5 and xa-9 when pathotyped using the national cultivar differentials and seven pathotypes on NIL differentials. Clear cut differences between the pathogen isolates originating from inbred rice varieties/cultures and from the hybrid rice varieties and their CMS, restorer and maintainer lines could not be observed. Virulence data obtained with NIL differentials revealed that all of them were compatible with the resistance genes Xa-3 and Xa-4. However, the pathotypes were incompatible with the genes, xa-5, Xa-10, xa-13 and Xa-21 suggesting the possibility of deploying them for enhancing the resistance of the rice hybrids tested.

CONCLUSIONS

The virulence pattern of all these 30 isolates was obtained using both the national cultivar differential set as well as the NIL differential set. These isolates were grouped into a total of four pathotypes, xa-1, xa-3, xa-5 and xa-9 when pathotyped using the national cultivar differentials (Table 1) (Figure 1). Pathotype xa-1 consisted of a maximum of 27 isolates. However, when all the 30 isolates were pathotyped using the NIL differentials, seven pathotypes, XA-1, XA-2, XA-5, XA-6, XA-8, XA-21, and XH-1 were detected (Table 2) (Figure 2). The pathotype XA-6 consisted of a maximum of 12 isolates.

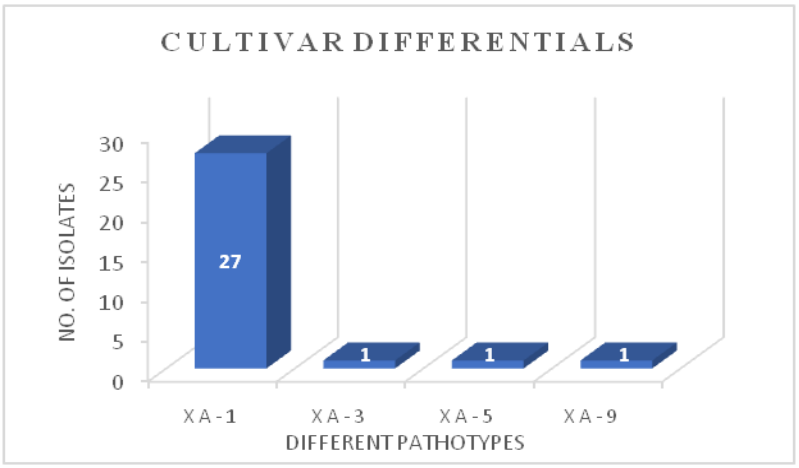

Figure 1: Pathotyping of Bacterial Blight Isolates using a set of National Cultivar Differentials.

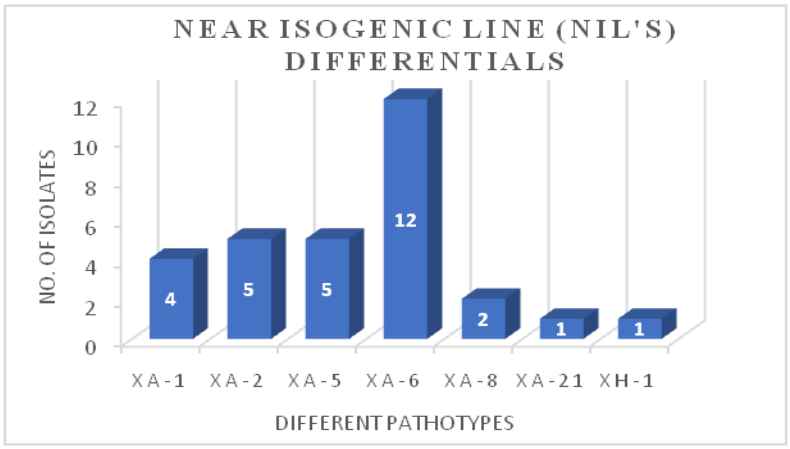

Figure 2: Pathotyping of Bacterial Blight Isolates using a set of Near-Isogenic Line Differentials.

Table: 1: Pathotyping of bacterial blight isolates (Dry season) obtained from hybrid rice cultivars of different parental origin using a set of national cultivar differentials. (Data are lesion length in cm, lesion length >5 cm is considered as susceptible)

S. No.	Isolate name	Classification	IR 8	IR 20	BJ 1	DV 85	Cempo Selak	Java 14	Reaction	Pathotype
1	CR 1014	Inbred culture	18.0	24.1	8.3	13.3	16.5	12.8	SSSSSS	xa-1
2	CR 749-20-2 (1)	Inbred culture	22.8	21.4	5.8	4.5	17.0	17.6	SSSRSS	xa-5
3	CR 749-20-2 (2)	Inbred culture	20.4	24.8	14.5	18.7	22.6	23.2	SSSSSS	xa-1
4	CR 679-2	Inbred culture	17.2	15.7	7.5	5.8	11.2	24.8	SSSSSS	xa-1
5	CR 839	Inbred culture	11.8	20.3	4.4	7.4	14.2	5.9	SSRSSS	xa-9
6	CR 2007	Inbred culture	26.2	25.2	13.6	19.4	28.8	21.5	SSSSSS	xa-1
7	IET 16645	Inbred culture	18.1	14.5	19.2	23.0	9.7	10.0	SSSSSS	xa-1
8	IR 62829A (1)	CMS line	14.0	14.6	5.5	11.6	20.3	18.4	SSSSSS	xa-1
9	IR 62829A (2)	CMS line	19.6	14.2	19.3	18.0	20.3	15.0	SSSSSS	xa-1
10	CRMS 31A	CMS line	14.7	19.6	12.0	13.7	15.6	11.3	SSSSSS	xa-1
11	CRMS 32A	CMS line	17.1	16.3	14.0	13.5	17.1	17.5	SSSSSS	xa-1
12	IR 62829B	Maintainer line	16.8	16.0	13.2	8.9	7.7	11.8	SSSSSS	xa-1
13	IR 53258	Restorer line	18.2	19.3	20.9	24.5	18.3	14.2	SSSSSS	xa-1
14	IR 42266	Restorer line	21.0	18.1	25.7	28.5	21.9	13.7	SSSSSS	xa-1
15	RH 10	Pusa hybrid	6.4	3.4	4.4	4.1	4.4	3.2	SRRRRR	xa-32
16	PA 6201	Proagro hybrid	18.0	18.8	25.3	32.1	24.5	24.9	SSSSSS	xa-1
17	HKRH 1008	Indo-American hybrid	14.4	9.1	14.5	13.9	14.7	11.0	SSSSSS	xa-1
18	MPH 525	Mahyco hybrid	22.0	19.4	22.1	18.7	23.5	19.9	SSSSSS	xa-1
19	PAC 89001	ITC hybrid	17.2	13.5	20.0	21.8	12.2	13.0	SSSSSS	xa-1
20	PHB 71 (1)	PAU hybrid	20.2	19.1	23.8	25.8	5.5	21.4	SSSSSS	xa-1
21	PHB 71 (2)	PAU hybrid	10.4	16.8	12.4	9.9	10.1	17.6	SSSSSS	xa-1
22	CRHR 1	CRRI hybrid	20.5	20.9	22.8	27.6	14.3	19.1	SSSSSS	xa-1
23	CRHR 4 (1)	CRRI hybrid	18.3	16.9	10.3	12.1	24.5	23.3	SSSSSS	xa-1
24	CRHR 4 (2)	CRRI hybrid	14.1	17.9	16.8	14.6	22.3	13.4	SSSSSS	xa-1
25	CRHR 4 (3)	CRRI hybrid	16.1	18.8	12.0	12.9	19.7	19.0	SSSSSS	xa-1
26	CRHR 5 (1)	CRRI hybrid	21.9	21.8	13.4	8.4	20.6	18.4	SSSSSS	xa-1
27	DRRH 1	DRR hybrid	22.1	18.4	9.8	8.4	15.7	15.6	SSSSSS	xa-1
28	MTURH 2020	Maruteru hybrid	20.7	19.0	10.5	6.3	14.2	14.8	SSSSSS	xa-1
29	NSD-2 (1)	NDUAT hybrid	14.8	16.2	11.4	17.5	14.4	7.7	SSSSSS	xa-1
30	NSD-2 (2)	NDUAT hybrid	18.7	28.5	11.4	12.5	21.3	29.1	SSSSSS	xa-1

Table 2: Pathotyping of bacterial blight isolates (Dry season) obtained from hybrid rice cultivars of different parental origin using a set of near-isogenic line differentials. (Data are lesion length in cm, lesion length >5 cm is considered as susceptible)

S. No.	Isolate name	Classification	IRBB 3	IRBB 4	IRBB 5	IRBB 7	IRBB 10	IRBB 13	IRBB 21	Reaction	Pathotype
1	CR 1014	Inbred culture	12.5	12.1	9.7	8.9	3.9	9.1	4.2	SSSSRSR	XA-6
2	CR 749-20-2 (1)	Inbred culture	10.1	10.2	13.5	11.8	13.3	11.4	3.8	SSSSSSR	XA-2
3	CR 749-20-2 (2)	Inbred culture	20.6	16.5	18.2	21.0	5.4	13.7	6.1	SSSSSSS	XA-1
4	CR 679-2	Inbred culture	9.4	10.5	9.1	10.9	3.6	6.9	4.6	SSSSRSR	XA-6
5	CR 839	Inbred culture	9.8	9.3	5.6	7.8	4.3	5.3	4.2	SSSSRSR	XA-6
6	CR 2007	Inbred culture	20.5	29.5	18.7	20.9	11.0	7.8	5.6	SSSSSSS	XA-1
7	IET 16645	Inbred culture	7.8	11.5	9.5	8.5	4.9	16.3	3.5	SSSSRSR	XA-6
8	IR 62829A (1)	CMS line	20.8	20.6	21.2	20.5	6.5	8.7	3.7	SSSSSSR	XA-2
9	IR 62829A (2)	CMS line	14.0	14.6	17.0	15.2	2.9	19.9	3.8	SSSSRSR	XA-6
10	CRMS 31A	CMS line	16.2	19.4	19.4	16.6	6.3	13.5	2.1	SSSSSSR	XA-2
11	CRMS 32A	CMS line	16.1	17.0	13.5	22.2	4.0	18.4	3.6	SSSSRSR	XA-6
12	IR 62829B	Maintainer line	19.7	22.3	21.3	20.7	9.4	25.5	6.4	SSSSSSS	XA-1
13	IR 53258	Restorer line	16.7	16.8	15.1	17.4	8.2	10.6	4.2	SSSSSSR	XA-2
14	IR 42266	Restorer line	19.2	24.9	26.2	21.1	4.1	16.9	8.4	SSSSRSS	XA-5
15	RH10	Pusa hybrid	4.9	4.7	3.3	4.2	4.0	4.2	2.8	RRRRRRR	XH-1
16	PA 6201	Proagro hybrid	13.5	26.8	21.6	18.7	4.0	16.0	4.9	SSSSRSR	XA-6
17	HKRH 1008	Indo-American hybrid	11.2	13.1	9.3	6.6	3.4	16.4	4.4	SSSSRSR	XA-6
18	MPH 525	Mahyco hybrid	14.6	19.3	18.1	17.0	3.6	4.1	2.9	SSSSRRR	XA-8
19	PAC 89001	ITC hybrid	19.6	26.3	22.6	19.2	3.7	12.5	5.1	SSSSRSS	XA-5
20	PHB 71 (1)	PAU hybrid	12.5	13.9	16.3	16.3	0.4	12.3	4.7	SSSSRSR	XA-6
21	PHB 71 (3)	PAU hybrid	5.5	5.7	7.7	5.7	3.5	6.2	3.6	SSSSRSR	XA-6
22	CRHR 1	CRRI hybrid	20.7	24.4	23.7	24.6	4.2	18.5	7.6	SSSSRSS	XA-5
23	CRHR 4 (1)	CRRI hybrid	15.9	13.4	4.1	12.6	4.5	10.1	10.5	SSRSRSS	XA-21
24	CRHR 4 (2)	CRRI hybrid	12.0	14.6	12.9	11.1	3.6	14.2	4.2	SSSSRSR	XA-6
25	CRHR 4 (3)	CRRI hybrid	13.3	13.5	11.5	13.7	3.8	16.6	6.6	SSSSRSS	XA-5
26	CRHR 5 (1)	CRRI hybrid	15.0	19.0	15.1	15.4	4.0	13.1	6.2	SSSSRSS	XA-5
27	DRRH 1	DRR hybrid	14.6	15.4	12.3	14.0	4.9	36.8	4.3	SSSSRSR	XA-6
28	MTURH 2020	Maruteru hybrid	10.2	10.8	12.3	9.8	6.4	14.2	4.1	SSSSSSR	XA-2
29	NSD-2 (1)	NDUAT hybrid	9.9	12.2	9.5	8.2	2.1	4.8	3.4	SSSSRRR	XA-8
30	NSD-2 (2)	NDUAT hybrid	19.5	20.6	19.2	20.0	10.3	13.4	7.7	SSSSSSS	XA-1

Clear cut differences between the pathogen isolates originating from inbred rice varieties/cultures and from the hybrid rice varieties and their CMS, restorer and maintainer lines could not be observed with the national cultivar set of differentials. Similar to the diversity showed by DNA fingerprint patterns, virulence analysis also exhibited a high level of diversity among the pathogen strains when the differential set consisting of near-isogenic lines carrying single resistance genes was used. Virulence data obtained with NIL differentials revealed that all the isolates were compatible with the resistance genes Xa-3 and Xa-4. However, the pathotypes were incompatible with the genes, xa-5, Xa-10, xa-13 and Xa-21 suggesting the possibility of deploying them for enhancing the resistance of the rice hybrids tested.

Incorporating resistance to bacterial blight in hybrid lines is a priority area. Chen et al. (2000) successfully deployed resistance gene Xa-21 into a most widely used restorer line Minghui 63 in China with a view to improve its resistance as well as to generate a resistant hybrid line.

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